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Combined epigallocatechin-3-gallate and resveratrol supplementation for 12 wk increases mitochondrial capacity and fat oxidation, but not insulin sensitivity, in obese humans: a randomized controlled trial^{1,2}

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ABSTRACT

Background: The obese insulin-resistant state is characterized by impairments in lipid metabolism. We previously showed that 3-d supplementation of combined epigallocatechin-3-gallate and resveratrol (EGCG+RES) increased energy expenditure and improved the capacity to switch from fat toward carbohydrate oxidation with a high-fat mixed meal (HFMM) test in men.

Objective: The present study aimed to investigate the longer-term effect of EGCG+RES supplementation on metabolic profile, mitochondrial capacity, fat oxidation, lipolysis, and tissue-specific insulin sensitivity.

Design: In this randomized double-blind study, 38 overweight and obese subjects [18 men; aged 38 ± 2 y; body mass index (kg/m^2): 29.7 ± 0.5] received either EGCG+RES (282 and 80 mg/d, respectively) or placebo for 12 wk. Before and after the intervention, oxidative capacity and gene expression were assessed in skeletal muscle. Fasting and postprandial (HFMM) lipid metabolism was assessed by using indirect calorimetry, blood sampling, and microdialysis. Tissue-specific insulin sensitivity was assessed by a hyperinsulinemic-euglycemic clamp with $[6,6\text{-}^2\text{H}_2]$ -glucose infusion.

Results: EGCG+RES supplementation did not affect the fasting plasma metabolic profile. Although whole-body fat mass was not affected, visceral adipose tissue mass tended to decrease after the intervention compared with placebo ($P\text{-time} \times \text{treatment} = 0.09$). EGCG+RES supplementation significantly increased oxidative capacity in permeabilized muscle fibers ($P\text{-time} \times \text{treatment} < 0.05$, $P\text{-EGCG+RES} < 0.05$). Moreover, EGCG+RES reduced fasting ($P\text{-time} \times \text{treatment} = 0.03$) and postprandial respiratory quotient ($P\text{-time} \times \text{treatment} = 0.01$) compared with placebo. Fasting and postprandial fat oxidation was not significantly affected by EGCG+RES ($P\text{-EGCG+RES} = 0.46$ and 0.38 , respectively) but declined after placebo ($P\text{-placebo} = 0.05$ and 0.03 , respectively). Energy expenditure was not altered ($P\text{-time} \times \text{treatment} = 0.96$). Furthermore, EGCG+RES supplementation attenuated the increase in plasma triacylglycerol concentrations during the HFMM test that was observed after placebo ($P\text{-time} \times \text{treatment} = 0.04$, $P\text{-placebo} = 0.01$). Finally, EGCG+RES had no effect on insulin-stimulated glucose disposal, suppression of endogenous glucose production, or lipolysis.

Conclusion: Twelve weeks of EGCG+RES supplementation increased mitochondrial capacity and stimulated fat oxidation compared with placebo, but this did not translate into increased tissue-specific insulin sensitivity in overweight and obese subjects. This trial was registered at clinicaltrials.gov as NCT02381145. *Am J Clin Nutr* 2016;104:215–27.

Keywords: insulin sensitivity, mitochondrial capacity, obesity, polyphenols, resveratrol

INTRODUCTION

The prevalence of obesity and related chronic diseases is continuously increasing (1). Insulin resistance is a major risk factor for the progression of obesity toward chronic metabolic diseases, including cardiovascular disease and type 2 diabetes (2). Reduced lipid storage capacity and impaired endocrine function of adipose tissue (3) and decreased mitochondrial capacity and accumulation of lipid intermediates in skeletal muscle (2, 4, 5) are closely associated with insulin resistance.

Current treatment strategies to control the progression of chronic diseases are mainly focused on lifestyle, pharmacologic, or surgical interventions. However, these interventions showed large interindividual variability in response (6), which underscores the need for additional strategies to optimize the prevention of obesity-associated metabolic disorders.

Polyphenols were identified as dietary ingredients with antioxidant properties decades ago. More recently, they were also implicated in the prevention of type 2 diabetes and cardiovascular

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² Supplemental Methods, Supplemental Material, Supplemental Tables 1 and 2, and Supplemental Figures 1 and 2 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

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diseases. As such, epigallocatechin-3-gallate (EGCG)⁶, which is most abundant in green tea, and resveratrol, which is present in grape skin, red wine, and peanuts, have been implicated in the prevention of body weight gain and insulin resistance in rodents fed obesogenic diets (7–9). In animal models of obesity, EGCG was shown to reduce the absorption of intestinal lipids and to increase lipid catabolism, possibly by inhibiting catechol-*O*-methyltransferase (10) or by activating AMP kinase (AMPK) (11). Resveratrol has been suggested to stimulate mitochondrial biogenesis by activating sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1 α) (7, 8).

Human studies have shown that supplementation with both EGCG (or EGCG-rich products) and resveratrol improved markers of insulin sensitivity [HOMA-IR, EGCG (12), resveratrol (13)], reduced plasma markers of oxidative stress (total antioxidative status) and systemic inflammation [TNF- α , EGCG (12), resveratrol (13)], stimulated postprandial fat oxidation [EGCG (14)], and reduced body weight [EGCG (15), resveratrol (16)]. However, conflicting data in humans have also been reported (17–19).

We recently postulated that a combination of polyphenols with distinct mechanisms of action may have additional and/or synergistic effects, which may result in physiologically relevant effects on fat oxidation. Indeed, we showed that supplementation of a specific combination of EGCG and resveratrol (EGCG+RES) for 3 d significantly increased resting and postprandial energy expenditure (EE) and resulted in a more pronounced increase in respiratory quotient (RQ) after a high-fat mixed meal (HFMM) compared with placebo in men (20). Importantly, these effects were not observed after single EGCG supplementation (21). Here, we investigated the effects of longer-term combined EGCG+RES supplementation on metabolic profile, skeletal muscle oxidative capacity, fat oxidation, lipolysis, and peripheral, hepatic, and adipose tissue insulin sensitivity in overweight and obese nondiabetic subjects.

METHODS

Subjects

Forty-two untrained (<3 h organized sports activities/wk), weight-stable (<2 kg body weight change 3 mo before inclusion) overweight and obese [BMI (in kg/m²) >25] white men ($n = 21$) and women ($n = 21$) aged between 20 and 50 y with normal glucose tolerance (fasting glucose <6.1 mmol/L, 2-h glucose <7.8 mmol/L), normal blood pressure (diastolic: 60–90 mm Hg; systolic: 100–140 mm Hg) were included in this study. Subjects were not allowed to donate blood or use any medication or supplements that might interfere with study outcomes for 3 mo before entering the study. Exclusion criteria were pregnancy, menopause, lactation, and any reported history of chronic inflammatory, cardiovascular, hepatic, pulmonary, renal,

or gastrointestinal disease. Intakes of caffeine (<600 mg/d), green tea (<3 cups/d), alcohol (<20 g/d), and grapes and peanuts (occasional consumption) had to be limited.

Study design and randomization

In this randomized, double-blind, placebo-controlled, parallel-intervention trial, subjects received either a combination of EGCG and RES supplements (EGCG+RES; 282 and 80 mg/d, respectively) or a placebo (partly hydrolyzed microcrystalline cellulose-filled capsules) for a period of 12 wk to assess effects of EGCG+RES supplementation on tissue-specific insulin sensitivity (primary outcome) and metabolic profile, skeletal muscle oxidative capacity, fat oxidation, and lipolysis (secondary outcomes). An independent researcher executed a block-wise randomization procedure and packed the supplements according to a computer-generated randomization plan (Microsoft Excel 2011 for Mac, Microsoft Corp.). The supplementation period started the day after the last baseline measurement in week 0 and was continued throughout measurements in week 12. Subjects were instructed to maintain their habitual lifestyle pattern throughout the study. In total, subjects were asked to visit the university 10 times for medical screening, 3 clinical investigation days (CIDs) before the start of supplementation (within 7 d), 3 control visits during the supplementation period, and 3 CIDs in the last week of supplementation (within 7 d). CIDs comprised skeletal muscle biopsies combined with dual-energy X-ray absorptiometry (CID1), an HFMM test (CID2), and a hyperinsulinemic-euglycemic clamp (CID3) and were separated by ≥ 1 d. Control visits for compliance, side effects, and to provide new capsules were scheduled in weeks 2, 4, and 8 of intervention. During weeks 0 and 12, subjects were asked to complete 3-d food records (2 weekdays, 1 weekend day) and during week 4 a 1-d food record (1 weekday) to monitor and control for changes in dietary habits. An experienced dietitian checked the food records and discussed these with the subjects in case of incomplete or missing information. Energy and nutrient intakes were analyzed by using the Dutch Food Composition data set (NEVO; National Institute for Public Health and the Environment, Ministry of Health, Welfare, and Sport, Netherlands).

CIDs

Two days before the CIDs, subjects were asked to refrain from intense physical activity and alcohol consumption. Meal intake during the evening before each CID was standardized per subject by providing the same meal. The first meal was provided ad libitum (740 ± 34 kcal, $48\% \pm 2\%$ of energy from fat), and subjects were asked to keep the portion size constant at all subsequent CIDs. After an overnight fast, subjects came to the university by car or public transport. Both before and after the intervention, a 2-step hyperinsulinemic-euglycemic clamp and an HFMM test were performed, and a skeletal muscle biopsy sample was collected. All procedures were performed while subjects were in a resting, half-supine position.

Dual-energy X-ray absorptiometry and skeletal muscle biopsy (CID1)

First, body composition was measured by dual-energy X-ray absorptiometry with the use of the 3-compartment model (Hologic Corp.). Next, skeletal muscle (*Vastus lateralis*) biopsy samples

⁶ Abbreviations used: AMPK, AMP activated kinase; CID, clinical investigation day; EE, energy expenditure; EGCG, epigallocatechin-3-gallate; EGCG+RES, epigallocatechin-3-gallate plus resveratrol; EGP, endogenous glucose production; FFA, free fatty acid; HFMM, high-fat mixed meal; NOGD, nonoxidative glucose disposal; OxPhos, oxidative phosphorylation; PGC1 α , peroxisome proliferator-activated receptor γ coactivator 1 α ; post, after intervention; pre, before intervention; R_d, rate of disappearance; RQ, respiratory quotient; SIRT1, sirtuin 1.

were taken under local anesthesia during fasting conditions before and after the 12-wk intervention period. One portion (~30 mg) was used for high-resolution respirometry (22), which allows for the determination of oxidative capacity *ex vivo* by quantifying the oxygen consumption with the use of the Oxygraph-2k (Oroboros Instruments). The other portion was directly frozen in isopentane and stored at -80°C until further analyses. Protein quantification of mitochondrial oxidative phosphorylation (OxPhos) complexes was performed by SDS-PAGE followed by Western blot analysis with the use of nitrocellulose membranes (Trans-blot Turbo transfer system; Bio-Rad). Blots were probed with Total OxPhos Antibody Cocktail (Mitosciences/Abcam) and a secondary Infrared dye680/700 conjugated donkey anti-mouse antibody (Licor/Invitrogen). Antigen-antibody complexes were visualized by using an Odyssey Infrared Imaging System (Licor Biosciences).

A detailed description for skeletal muscle microarray analysis and lipid composition is provided in the **Supplemental Methods**. Briefly, for microarray analysis, 100 ng intact total RNA was extracted from skeletal muscle biopsy samples by using the Trizol method (Qiagen) and processed by applying the GeneChip WT Plus Reagent Kit and Human Transcriptome Array 2.0 GeneChips (Affymetrix) according to the manufacturer's instructions. Functional data analysis was based on a false-discovery rate q -value <0.2 on the filtered data set [IQR >0.2 (log2), intensity >20 , >5 arrays, >5 probes/gene].

For the determination of lipid composition, total lipids were extracted after lyophilization of skeletal muscle tissue. Diacylglycerol and triacylglycerol were separated by thin-layer chromatography and fatty acid profiles were determined on an analytic gas chromatograph, as described previously (5).

Microdialysis (CID2)

Four microdialysis probes (CMA 60; CMA Microdialysis) were inserted under local anesthesia, 2 in the medial portion of the gastrocnemius muscle of both legs and 2 in the subcutaneous adipose tissue 6–8 cm left and right from the umbilicus in male subjects ($n = 17$; age: 40.1 ± 2.0 y; BMI: 30.0 ± 0.8 ; HOMA-IR: 2.1 ± 0.4), as described previously (23). After insertion, 90 min were allowed for tissue recovery from the insertion trauma. Throughout the HFMM test (CID2), in each tissue one probe was perfused at $0.3 \mu\text{L}/\text{min}$ with Ringer solution to collect microdialysate for analyses of glycerol, glucose, pyruvate, and lactate concentrations by means of CMA enzymatic assay kits on a CMA 600 microdialysis analyzer.

The contralateral probe was perfused at $5.0 \mu\text{L}/\text{min}$ with Ringer solution, supplemented with 50 mmol ethanol/L, to assess the ethanol outflow-to-inflow ratio as an indicator of local blood flow. Ethanol concentrations were measured spectrophotometrically at 340 nm by using a standard ethanol assay kit (Boehringer).

HFMM test (CID2)

After inserting a cannula into the antecubital vein, substrate oxidation was measured for 30 min under fasting conditions ($t = 0$) and for 4 h after the ingestion of a liquid HFMM (625 kcal, 61% of energy from fat, 33% of energy from carbohydrate, 6% of energy from protein), which was consumed within 5 min at $t = 0$. Blood samples were taken under fasting ($t = 0$ min) and post-

prandial ($t = 30, 60, 90, 120, 150, 180, 210$, and 240 min) conditions. EE and substrate oxidation were measured by indirect calorimetry by using the open-circuit ventilated hood system (OmniCal; Maastricht University) and were calculated according to the formulas of Weir (24) and Frayn (25), respectively.

Hyperinsulinemic-euglycemic clamp (CID3)

A 2-step hyperinsulinemic-euglycemic clamp with $[6,6-^2\text{H}_2]$ -glucose infusion (tracer; Cambridge Isotope Laboratories) was performed to assess the rate of disappearance (R_d), nonoxidative glucose disposal (NOGD), and endogenous glucose production (EGP) (22). First, a cannula was inserted into the antecubital vein. A second cannula was inserted into a superficial dorsal hand vein for the sampling of arterialized blood (by using a hot box with air circulating at $\sim 50^{\circ}\text{C}$). After the administration of a bolus injection of $2.4 \text{ mg } [6,6-^2\text{H}_2]\text{-glucose}/\text{kg}$, a continuous $[6,6-^2\text{H}_2]\text{-glucose}$ infusion was started at $0.04 \text{ mg}/(\text{kg} \times \text{min})$ and continued throughout the measurement. After 2 h, insulin infusion was started at $10 \text{ mU}/(\text{m}^2 \times \text{min})$ for 2 h, followed by $40 \text{ mU}/(\text{m}^2 \times \text{min})$ insulin for the last 2 h to suppress lipolysis and EGP. By a variable coinfusion of a 20% glucose solution, enriched to $1.92 \text{ mg tracer}/\text{mL}$, blood glucose concentrations were maintained at $5.0 \text{ mmol}/\text{L}$. During the last 30 min of each insulin-infusion step [$0, 10$, and $40 \text{ mU}/(\text{m}^2 \times \text{min})$], blood samples were collected and substrate oxidation was measured by using indirect calorimetry [described in the section entitled “HFMM test (CID2)"] to assess glucose kinetics. Kinetics of R_d and NOGD were calculated during 0 and $40 \text{ mU}/(\text{m}^2 \times \text{min})$ insulin infusion, respectively, as absolute increases between these steps [$\Delta \mu\text{mol}/(\text{kg} \times \text{min})$], whereas calculations for insulin-mediated suppression of EGP and free fatty acids (FFAs) were performed during 0 and $10 \text{ mU}/(\text{m}^2 \times \text{min})$ insulin infusion, respectively, as relative suppression during 10 compared with $0 \text{ mU}/(\text{m}^2 \times \text{min})$ (%).

Biochemistry

Blood was collected into prechilled tubes and centrifuged ($1000 \times g$; 10 min ; 4°C) and plasma was snap-frozen in liquid nitrogen and stored at -80°C until analyses. To check for treatment compliance, plasma epigallocatechin-3-gallate and resveratrol concentrations were determined by liquid chromatography–mass spectrometry (for details, see Supplemental Methods). In brief, for the measurement of free EGCG, $500 \mu\text{L}$ plasma was combined with the same volume of an ascorbic acid/EDTA stabilizing buffer. For analyses, an internal standard was added, followed by a $2 \times$ liquid-liquid extraction. Total resveratrol and total dihydro-resveratrol were measured as the sum of aglycone and conjugated forms (β -glucuronidase digestion required) after the addition of labeled internal standard by a liquid-liquid extraction. After centrifugation, an aliquot of the organic phase was evaporated to dryness, re-dissolved in injection solvent, and analyzed by using liquid chromatography–mass spectrometry systems. The isotopic enrichment of plasma glucose was determined by electron ionization gas chromatography–mass spectrometry and expressed as the tracer-to-tracee ratio for steady state calculations of R_d , NOGD, and EGP, as described previously (22).

Plasma glucose, lactate, FFA, triacylglycerol, total cholesterol and HDL-cholesterol concentrations were determined with an automated spectrophotometer (ABX Pentra 400 autoanalyzer;

Horiba ABX) by using enzymatic colorimetric kits. LDL-cholesterol concentrations were calculated by the Friedewald equation (26). Plasma glycerol concentrations were measured with an enzymatic assay (Enzytec Glycerol; Roche Biopharm) automated on a Cobas Fara spectrophotometric autoanalyzer (Roche Diagnostics). Circulating plasma concentrations of insulin, adiponectin, and leptin were analyzed by using commercially available radioimmunoassay kits (Human Insulin-specific Radioimmunoassay, Human Adiponectin Radioimmunoassay, and Human Leptin Radioimmunoassay; Millipore Corporation). Plasma concentrations of inflammatory markers (IL-6, IL-8, and TNF- α) were determined by using a multiplex ELISA (Human ProInflammatory II 4-Plex Ultra-Sensitive Kit; Meso Scale Diagnostics).

Supplements

The supplements were commercially available and were provided by Pure Encapsulations, Inc. All of the capsules were manufactured, tested, and checked in accordance to the standards of the European Union's Good Manufacturing Practices requirements.

EGCG capsules contained 94% epigallocatechin-3-gallate (141 mg/capsule, lot 3570112) and resveratrol capsules contained 20% trans-resveratrol (40 mg trans-resveratrol in *Polygonum cuspidatum* extract/capsule, lot 1611011). The doses of the supplements were based on previous study outcomes, in which these doses altered markers of substrate and energy metabolism [Timmers et al. (13): 150 mg resveratrol/d; Most et al. (20): 200 mg resveratrol/d, 282 mg EGCG/d]. Higher concentrations appear to have no (additional) benefit [Poulsen et al. (17): 1500 mg resveratrol/d; Brown et al. (19): 800 mg EGCG/d]. Two lots of placebo capsules (microcrystalline cellulose: lots 7150712 and 7160712) were used for blinding. Placebo capsules were encapsulated identically to the active supplements and filled with microcrystalline cellulose and tan/brown and pink/red powder, respectively, to blind both supplements. Capsules were packed into white opaque boxes, labeled per subject without indication of the content. One capsule of each supplement (EGCG and resveratrol or both blinded placebo capsules) was ingested during breakfast and dinner. This regimen was maintained until the last measurement. On CIDs, subjects ingested the capsules before arrival at the university (between 0700 and 0800 h). After completion of the study, returned capsules were counted for compliance and the stability of polyphenols was assessed by measuring EGCG and resveratrol via HPLC in capsules stored for 3 mo, according to the manufacturer's recommendations. The supplements were considered safe and well tolerated (20).

Study approval

All of the subjects gave written informed consent for participation in this study, which was reviewed and approved by the local medical ethics committee of Maastricht University Medical Center. This trial was registered at clinicaltrials.gov (NCT02381145). All procedures were conducted according to the Declaration of Helsinki.

Statistical analysis

The sample size ($n = 42$) was calculated to detect a physiologically relevant change in insulin sensitivity of 20%, with an SD of 4%, a power of 80%, assuming a significance level of $\alpha = 0.05$ ($n = 34$), and a drop-out rate of 20% ($n = 8$). All of the data

are expressed as means \pm SEMs. Data for the HFMM test are expressed as AUCs to define the total measurement period during this test (fasting and postprandial) and as incremental AUCs to define specifically the postprandial response (diet-induced effect compared with fasting), both calculated by the trapezoid method. Variables were tested for normality by using Shapiro-Wilk test and showed a normal distribution. Differences in subjects' characteristics at baseline were tested by Student's unpaired t test. Data were analyzed by using a 2-factor repeated-measures ANOVA, with time [before intervention (pre) and after intervention (post)] and treatment (placebo, EGCG+RES) as factors. $P < 0.05$ was considered to be significant, and $P < 0.10$ was considered to show a trend. In case of a significant time \times treatment interaction (P -time \times treatment), post hoc analyses with Bonferroni correction were applied to identify significant within-group effects (P -EGCG+RES, P -placebo). In case of clinically relevant baseline differences between treatment groups (for fasting insulin, IL-6, mitochondrial capacity of state 3 respiration, and protein expression of OxPhos complexes III and V; for all, $>20\%$ baseline difference), the baseline value was included as a covariate in univariate ANOVA, with the change over time as a dependent variable and treatment as a between-subjects factor. In the ANOVA model, no interactions between sex and the primary and secondary outcomes were observed. Statistical analysis was performed by using SPSS 19.0 (IBM Corporation) for Macintosh.

RESULTS

Subject characteristics and plasma biochemistry

Forty-two overweight and obese men and women volunteered to participate in this study (August 2012–December 2013). In total, 4 subjects (3 men, 1 women) did not complete the study due to traveling abroad ($n = 1$), re-employment ($n = 1$), or noncompliance with respect to supplementation or changing lifestyle ($n = 2$). Characteristics of the 38 subjects (18 men, 20 women) who completed the study are summarized in **Table 1**. There were no significant differences between the EGCG+RES ($n = 18$; 8 men) and placebo ($n = 20$; 10 men) groups with respect to baseline characteristics or self-reported moderate or intense physical activity (EGCG+RES: 1.4 ± 0.3 h/wk; placebo: 1.6 ± 0.2 h/wk; $P = 0.78$).

Compliance was confirmed by counting returned supplements ($<3\%$ of supplements were returned). Supplements were well tolerated, and no adverse events were reported. Importantly, plasma concentrations of EGCG, resveratrol, and dihydro-resveratrol were significantly increased in all subjects who had received EGCG+RES (**Table 2**), which indicated compliance to the supplementation regimen. No significant changes in fasting plasma concentrations of glucose, insulin, lactate, glycerol, FFAs, or triacylglycerol were observed (Table 2, **Figure 1A–F**). Likewise, fasting plasma concentrations of leptin, adiponectin, and the inflammatory markers IL-6, IL-8, and TNF- α were not significantly affected by the intervention (Table 2). Furthermore, fasting plasma concentrations of total cholesterol, HDL cholesterol, and LDL cholesterol were not significantly changed after 12 wk of EGCG+RES supplementation compared with placebo. However, the ratio of total cholesterol to HDL cholesterol, a marker for myocardial infarction risk (27), tended to

TABLE 1
Subject characteristics¹

	Placebo (n = 20)	EGCG+RES (n = 18)	P (t test)
Age, y	38.7 ± 2.2	36.1 ± 2.2	0.43
BMI, kg/m ²	29.5 ± 0.7	29.9 ± 0.6	0.67
Waist-hip ratio	0.87 ± 0.02	0.87 ± 0.02	0.78
Systolic blood pressure, mm Hg	114 ± 2	117 ± 2	0.27
Diastolic blood pressure, mm Hg	76 ± 2	76 ± 2	0.86
Fasting plasma glucose, mmol/L	5.10 ± 0.08	5.19 ± 0.09	0.44
2-h Glucose, mmol/L	5.38 ± 0.21	5.34 ± 0.24	0.92
HbA1c, %	5.15 ± 0.06	5.12 ± 0.06	0.76
Hemoglobin, mmol/L	8.9 ± 0.1	8.6 ± 0.2	0.22
Hematocrit, L/L	0.43 ± 0.01	0.39 ± 0.02	0.18
Creatinine, μmol/L	79.5 ± 3.7	74.2 ± 2.7	0.29
ALT, U/L	27.9 ± 4.4	26.9 ± 2.2	0.86

¹Values are means ± SEMs. Data were analyzed by using a Student's unpaired *t* test. *P* < 0.05 was considered significant for the difference between placebo and EGCG+RES groups. ALT, alanine aminotransferase; EGCG+RES, epigallocatechin-3-gallate plus resveratrol; HbA1c, glycated hemoglobin; 2-h Glucose, plasma glucose concentration 2 h after oral glucose ingestion.

decrease after EGCG+RES supplementation but not after placebo (*P*-time × treatment = 0.03, *P*-EGCG+RES = 0.09, *P*-placebo = 0.13; Table 2). Adjustments for clinically relevant differences in baseline values (fasting insulin and IL-6) did not alter the outcome of the analysis (>20% baseline difference).

EGCG+RES did not alter food intake and body composition

Food intake, assessed by using self-reported 3-d food records, did not change after supplementation in either group (EGCG

+RES: 2092 ± 200 kcal/d, 45% ± 1% of energy from carbohydrate, 39% ± 1% of energy from fat, 15% ± 1% of energy from protein; placebo: 1982 kcal/d, 45% ± 2% of energy from carbohydrate, 38% ± 2% of energy from fat, 16% ± 1% of energy from protein). Body weight, body fat percentage, and body fat distribution (upper and lower body fat mass) were not significantly altered by EGCG+RES supplementation (Table 3). Interestingly, however, there was a tendency toward a differential effect on visceral adipose tissue mass, with a tendency toward a decrease after EGCG+RES compared with placebo (*P*-time × treatment = 0.09).

EGCG+RES increased skeletal muscle oxidative capacity

Next, we investigated whether skeletal muscle oxidative capacity increased after 12 wk of EGCG+RES supplementation, because we previously showed that resveratrol supplementation for 30 d improved mitochondrial capacity (13). Therefore, ex vivo mitochondrial respiration with the use of different substrate combinations was determined in isolated human permeabilized skeletal muscle fibers (22).

Basal respiration [placebo (pre): 6.95 ± 0.96; placebo (post): 3.93 ± 1.03; EGCG+RES (pre): 3.67 ± 1.17; and EGCG+RES (post): 4.87 ± 1.04 pmol O₂ · mg muscle⁻¹ · s⁻¹] increased by ~10-fold by the addition of ADP (state 3 respiration over basal respiration, with malate and glutamate as substrates; data not shown). This indicates that the permeabilized fibers are reactive to ADP and the mitochondrial ADP transport is intact and functioning. ADP-stimulated complex I-linked respiration with the addition of malate + glutamate was not affected by EGCG+RES supplementation (data not shown). In addition, respiration with a fatty acid substrate, assessed by input of malate + octanoyl-carnitine, was not significantly affected by EGCG+RES (*P*-time ×

TABLE 2
Fasting plasma biochemistry before and after 12 wk of supplementation with EGCG+RES or placebo¹

	Placebo (n = 20)		EGCG+RES (n = 18)		<i>P</i>		
	Week 0	Week 12	Week 0	Week 12	Time	Treatment	Time × treatment
EGCG, nmol/L	<5.45	<5.45	<5.45	32.7 ± 13.1*			
Resveratrol, nmol/L	<43.8	<43.8	<43.8	1200.5 ± 220*			
Dihydro-resveratrol, nmol/L	<43.4	<43.4	<43.4	833.8 ± 121.6*			
Glucose, mmol/L	5.0 ± 0.1	5.1 ± 0.1	5.1 ± 0.1	5.1 ± 0.1	0.75	0.61	0.69
Insulin, mU/L	10.1 ± 1.5	10.0 ± 1.0	8.1 ± 1.0	7.4 ± 0.6	0.54	0.10	0.67
HOMA-IR	2.3 ± 0.4	2.2 ± 0.2	1.8 ± 0.2	1.7 ± 0.1	0.57	0.12	0.74
FFAs, μmol/L	553 ± 33	533 ± 43	523 ± 24	555 ± 35	0.89	0.92	0.37
Triacylglycerol, mmol/L	1.29 ± 0.16	1.52 ± 0.16	1.61 ± 0.23	1.61 ± 0.24	0.13	0.46	0.17
Cholesterol, mmol/L	5.9 ± 0.3	5.8 ± 0.3	6.0 ± 0.2	5.8 ± 0.2	0.13	0.81	0.49
HDL cholesterol, mmol/L	1.23 ± 0.07	1.20 ± 0.07	1.27 ± 0.08	1.29 ± 0.09	0.66	0.57	0.14
LDL cholesterol, mmol/L	4.40 ± 0.27	4.30 ± 0.27	4.45 ± 0.22	4.20 ± 0.16	0.06	0.94	0.39
Cholesterol:HDL cholesterol	5.09 ± 0.34	5.27 ± 0.43	5.16 ± 0.42	4.94 ± 0.42	0.94	0.82	0.03
Adiponectin, μg/mL	9.01 ± 0.85	9.64 ± 1.05	8.74 ± 0.82	8.99 ± 0.87	0.15	0.93	0.52
Leptin, ng/mL	23.4 ± 4.2	22.7 ± 4.1	19.8 ± 2.4	18.2 ± 2.4	0.28	0.41	0.65
IL-6, pg/mL	0.96 ± 0.17	0.83 ± 0.11	0.77 ± 0.07	0.85 ± 0.13	0.93	0.68	0.25
IL-8, pg/mL	9.25 ± 0.61	9.82 ± 0.86	9.80 ± 0.93	9.19 ± 0.70	0.98	0.97	0.26
TNF-α, pg/mL	2.84 ± 0.21	3.15 ± 0.42	2.69 ± 0.18	2.63 ± 0.15	0.34	0.35	0.20

¹Values are means ± SEMs. Data were analyzed by using 2-factor repeated-measures ANOVA, with time (pre, post) and treatment (placebo, EGCG+RES) as factors. For EGCG, resveratrol, and dihydro-resveratrol, 2.5, 10, and 10 ng/mL, respectively, were detection limits. *P* < 0.05 was considered significant. No baseline differences were observed (Student's *t* test). *, In case of a significant time × treatment interaction, post hoc analyses with Bonferroni correction were applied to identify significant within-group effects. Except for polyphenol concentrations, no significant within-group effects were found. EGCG, free plasma epigallocatechin-3-gallate; EGCG+RES, epigallocatechin-3-gallate plus resveratrol; FFA, free fatty acid; post, after intervention; pre, before intervention; Resveratrol, plasma total resveratrol.

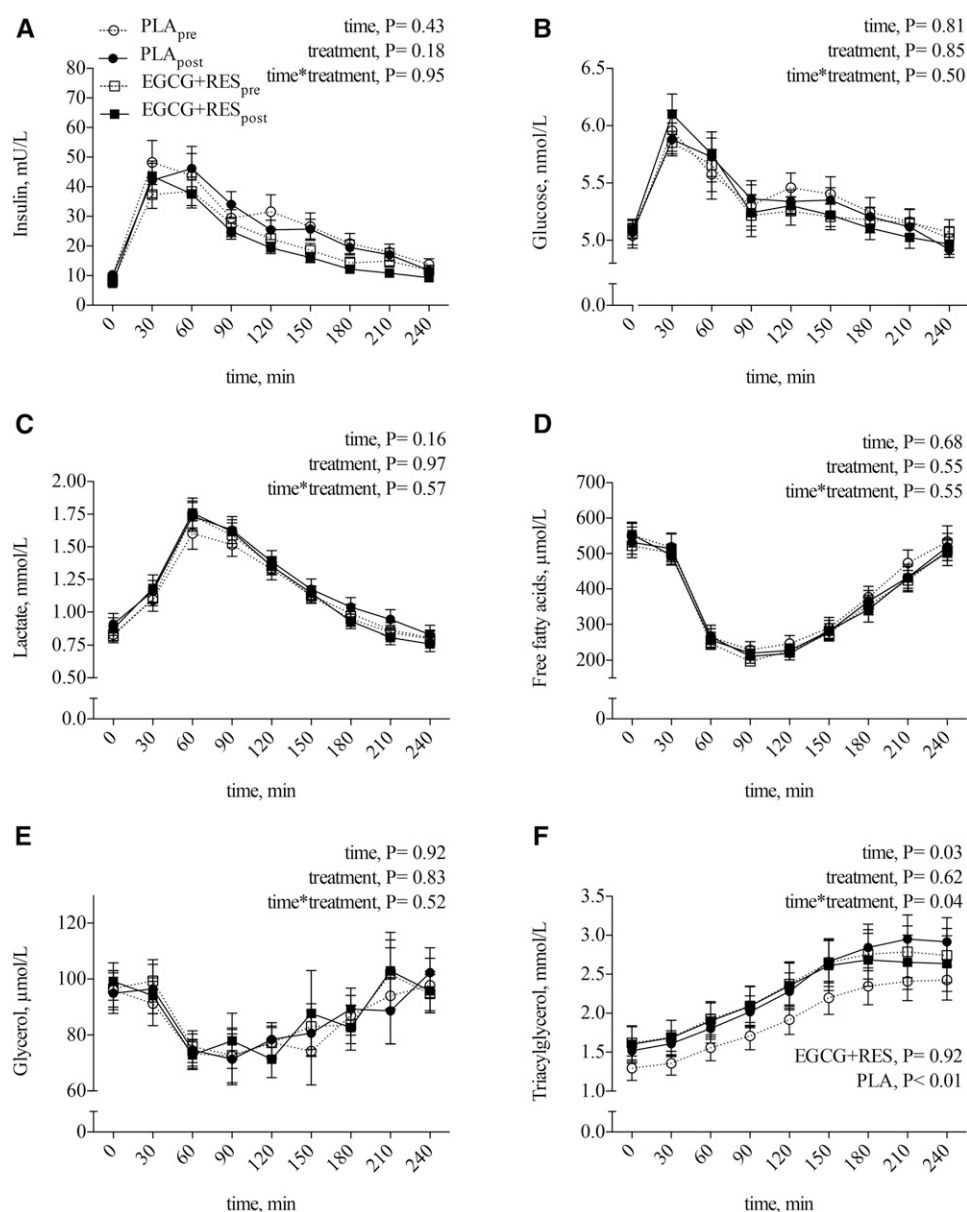


FIGURE 1 Fasting and postprandial insulin and metabolite concentrations before and after intervention. Values are means \pm SEMs; $n = 38$. Plasma insulin (A) and metabolite (B–F) concentrations are shown after an overnight fast (at 0 min) and after an HFMM (from 0 to 240 min). Fasting values ($t = 0$), AUCs during the HFMM test (calculated from $t = 0$ –240 min), and incremental AUCs were analyzed by using 2-factor repeated-measures ANOVA, with time [P -time (pre, post)] and treatment [P -treatment (placebo, EGCG+RES)] as factors. $P < 0.05$ was considered significant. No time \times treatment interactions were observed on fasting concentrations and incremental AUCs. Reported P values refer to the respective AUCs. In case of a significant time \times treatment interaction (P -time \times treatment), post hoc analyses with Bonferroni correction were applied to identify significant within-group effects (EGCG+RES, P ; placebo, P). EGCG+RES, epigallocatechin-3-gallate plus resveratrol; HFMM, high-fat mixed meal; PLA, placebo; post, after intervention; pre, before intervention.

treatment = 0.19; **Figure 2A**). Interestingly, however, we found that mitochondrial respiration on the electron input of both complexes I and II (state 3, complex I and II), assessed by the addition of succinate after malate + glutamate (MGS) or after malate + octanoyl-carnitine + glutamate (MOGS), increased in the EGCG+RES group compared with the placebo group after 12 wk of supplementation [state 3 respiration (MGS): P -time \times treatment = 0.01, P -EGCG+RES = 0.01, P -placebo = 0.26; state 3 respiration (MOGS): P -time \times treatment = 0.05, P -EGCG+RES < 0.01, P -placebo = 0.45; **Figure 2B, C**]. Despite randomization, there was a difference in state 3 mitochondrial respiration at base-

line between groups (P -pre = 0.01), which was inversely correlated to the change in this variable over time ($r = -0.57$, $P < 0.01$). For this reason, baseline state 3 respiration was included as a covariate in the analysis.

Furthermore, maximal mitochondrial respiration, as determined by titration of the chemical uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, increased after EGCG+RES supplementation compared with placebo (P -time \times treatment = 0.01, P -EGCG+RES = 0.01, P -placebo = 0.31; **Figure 2D**). Mitochondrial proton leak, which was assessed by the addition of the ATP synthase inhibitor oligomycin, was not significantly

TABLE 3Body composition before and after 12 wk of supplementation with EGCG+RES or placebo¹

	Placebo (<i>n</i> = 20)		EGCG+RES (<i>n</i> = 18)		<i>P</i>		
	Week 0	Week 12	Week 0	Week 12	Time	Treatment	Time × treatment
Body weight, kg	88.3 ± 2.8	88.6 ± 2.9	92.4 ± 3.6	92.0 ± 3.8	0.96	0.46	0.41
Body fat, kg	26.7 ± 1.9	26.5 ± 1.9	29.4 ± 1.4	29.1 ± 1.6	0.32	0.36	0.85
Body fat, %	30.1 ± 1.9	29.9 ± 1.8	31.9 ± 1.4	31.5 ± 1.4	0.11	0.55	0.84
Lean mass, kg	59.5 ± 2.5	59.6 ± 2.5	61.2 ± 3.0	61.1 ± 3.2	0.77	0.67	0.73
Upper body fat, kg	13.1 ± 1.0	12.9 ± 1.0	14.0 ± 0.7	13.8 ± 0.7	0.33	0.49	0.93
Lower body fat, kg	9.4 ± 0.9	9.4 ± 0.9	11.0 ± 0.8	10.8 ± 0.9	0.24	0.32	0.51
Visceral adipose tissue, kg	0.48 ± 0.04	0.50 ± 0.05	0.44 ± 0.05	0.41 ± 0.04	0.88	0.39	0.09

¹Values are means ± SEMs. Data were analyzed by using 2-factor repeated-measures ANOVA, with time [*P*-time (pre, post)] and treatment [*P*-treatment (placebo, EGCG+RES)] as factors. *P* < 0.05 was considered significant. No baseline differences were observed (Student's *t* test). In case of a significant time × treatment interaction (*P*-time × treatment), post hoc analyses with Bonferroni correction were applied to identify significant within-group effects. No significant within-group effects were found. EGCG+RES, epigallocatechin-3-gallate plus resveratrol; post, after intervention; pre, before intervention.

affected by the intervention (state 4o, *P*-time × treatment = 0.10; Figure 2E).

EGCG+RES increased oxidative metabolism pathways at the transcriptional and translational levels

To identify pathways that may underlie the EGCG+RES-induced improvement in mitochondrial capacity, we next determined whether protein expression of OxPhos complexes in skeletal muscle was altered by EGCG+RES. We found an EGCG+RES-induced increase in complexes III (*P*-time × treatment = 0.03, *P*-EGCG+RES = 0.04, *P*-placebo = 0.35) and V (*P*-time × treatment < 0.01, *P*-EGCG+RES < 0.01, *P*-placebo = 0.29) compared with placebo (Figure 2G, H). Despite randomization, there was a difference in complexes III and V at baseline between groups (>20% baseline difference). Adjustment for these baseline values did not alter the outcome of the analysis. On the basis of these findings, we performed microarray analysis on skeletal muscle biopsy samples (*Vastus lateralis*) collected before and after 12 wk of EGCG+RES or placebo treatment. Indeed, gene set enrichment analysis indicated that the most upregulated pathways after EGCG+RES supplementation were related to the citric acid cycle and respiratory electron transport chain, whereas pathways related to carbohydrate metabolism were upregulated in the placebo group (Figure 3, Supplemental Table 1). Taken together, these data indicate that the increased mitochondrial capacity after EGCG+RES supplementation is accompanied by changes at the transcriptional and translational levels.

EGCG+RES affects fat oxidation without changes in EE

Next, we investigated whether the improved skeletal muscle oxidative capacity after 12 wk of EGCG+RES supplementation translates into whole-body effects on fasting substrate oxidation. Indeed, EGCG+RES supplementation significantly affected fasting substrate oxidation, as reflected by no change in fasting RQ after EGCG+RES and a significant increase in the placebo group [EGCG+RES (pre): 0.803 ± 0.009; EGCG+RES (post): 0.785 ± 0.016; placebo (pre): 0.784 ± 0.009; placebo (post): 0.821 ± 0.015; *P*-time × treatment = 0.03, *P*-EGCG+RES = 0.31, *P*-placebo = 0.04] (Figure 4A), without effects on EE (Figure 4B). More specifically, an increase in fasting carbohydrate oxidation was observed after placebo but not after EGCG+RES (*P*-time × treatment = 0.05, *P*-EGCG+RES = 0.44,

P-placebo = 0.04) supplementation. Furthermore, EGCG+RES did not change fasting fat oxidation, whereas there was a significant decrease in the placebo group after 12 wk of supplementation compared with week 0 (*P*-time × treatment = 0.06, *P*-EGCG+RES = 0.46, *P*-placebo = 0.05; Figure 4C, D).

EGCG+RES affects triacylglycerol concentrations but did not change postprandial responses of substrate oxidation and local lipolysis

To examine EGCG+RES-induced effects on postprandial substrate utilization and EE, we performed an HFMM test (625 kcal, 61% of energy from fat). More specifically, we determined whole-body, adipose tissue, and skeletal muscle substrate metabolism in the postprandial state by combining plasma blood sampling, indirect calorimetry, and microdialysis.

The effects of 12 wk of EGCG+RES supplementation on substrate oxidation in the absence of changes in EE in the fasting state were maintained throughout the postprandial period (Figure 4E–H). More specifically, postprandial fat oxidation (AUC) was reduced in the placebo group in week 12 compared with week 0 (*P*-placebo = 0.03), whereas no significant effect of EGCG+RES was observed (*P*-EGCG+RES = 0.38; Figure 4H). In addition, the intervention-induced changes in EGCG, resveratrol, and dihydro-resveratrol plasma concentrations did not significantly correlate with EGCG+RES-induced effects on fat oxidation (data not shown).

The postprandial responses after meal intake (incremental AUC) of RQ, EE, carbohydrate, and fat oxidation as well as the postprandial responses of plasma metabolites and insulin were not significantly affected by the intervention (Figure 4I–L and Figure 1A–F, respectively).

Importantly, however, plasma triacylglycerol concentrations during the HFMM test were significantly increased in the placebo group after 12 wk but not in the EGCG+RES group [placebo (pre): AUC = 440 ± 56; placebo (post): AUC = 531 ± 62; EGCG+RES (pre): AUC = 527 ± 59; and EGCG+RES (post): AUC = 530 ± 66 mmol × 4 h/L; *P*-time × treatment = 0.04, *P*-EGCG+RES = 0.92, *P*-placebo < 0.01] (Figure 1F).

Skeletal muscle and abdominal subcutaneous adipose tissue substrate metabolism and blood flow were investigated in men with the use of microdialysis. Adipose tissue and skeletal muscle ethanol outflow-to-inflow ratios, which indicate local blood flow,

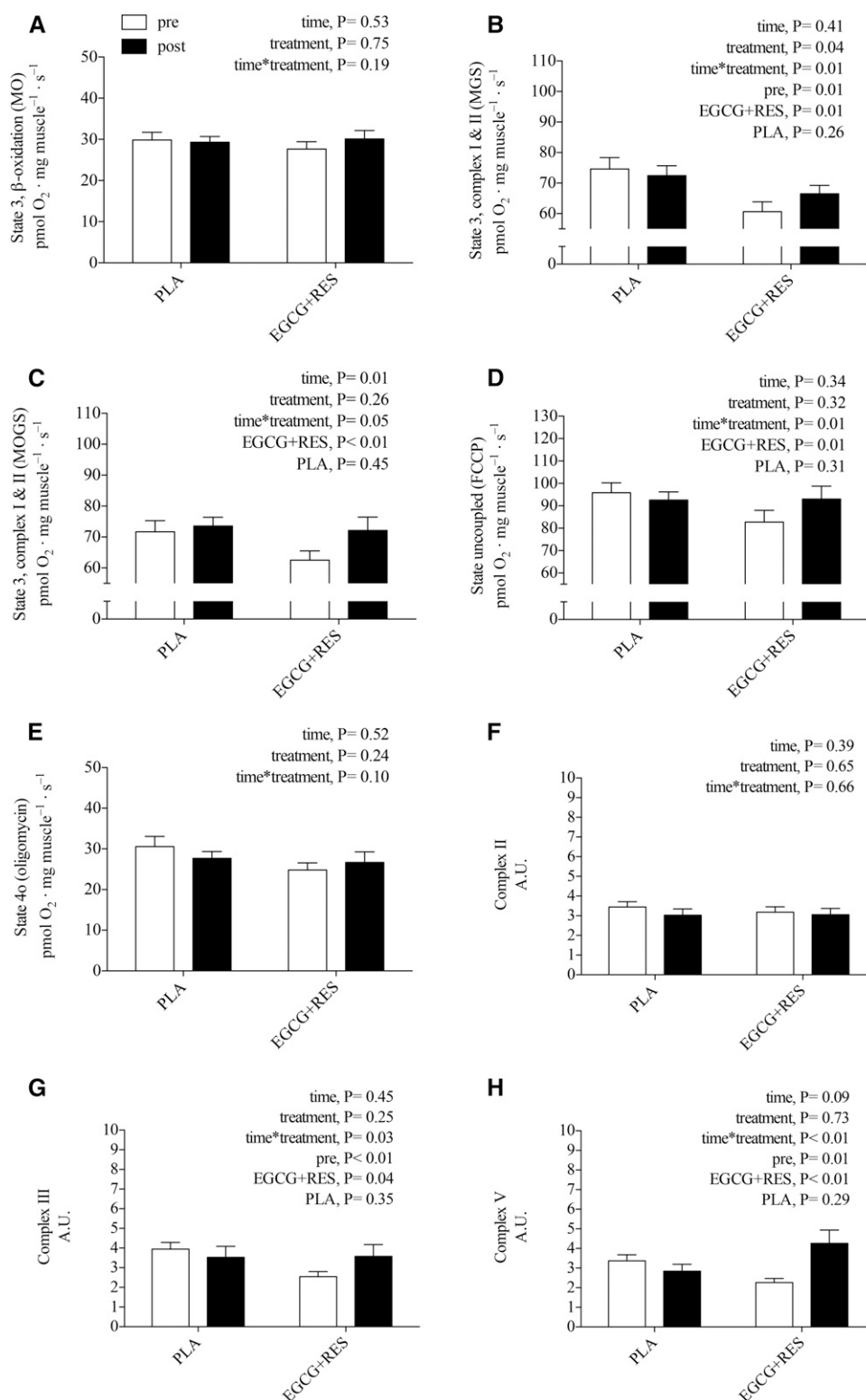


FIGURE 2 Skeletal muscle oxidative capacity and OxPhos protein content before and after the intervention. Values are means \pm SEMs; $n = 29$. Oxidative capacity (A–E) was assessed by means of ex vivo respirometry on skeletal muscle (*Vastus lateralis*), isolated after an overnight fast. In aliquots, protein contents of OxPhos complexes were analyzed by Western blots (F–H). Variables were analyzed by using 2-factor repeated-measures ANOVA, with time [P -time (pre, post)] and treatment [P -treatment (placebo, EGCG+RES)] as factors. $P < 0.05$ was considered significant. Significant baseline differences were identified by Student's unpaired t test [P (pre)]; and in case of a significant time \times treatment interaction (P -time \times treatment), post hoc analyses with Bonferroni correction were applied to identify significant within-group effects (EGCG+RES, P ; placebo, P). A.U., arbitrary units; EGCG+RES, epigallocatechin-3-gallate plus resveratrol; FCCP, carbonyl cyanide p -trifluoromethoxyphenylhydrazone; G, glutamate; M, malate; O, octanoyl-carnitine; OxPhos, oxidative phosphorylation; PLA, placebo; post, after intervention; pre, before intervention; S, succinate.

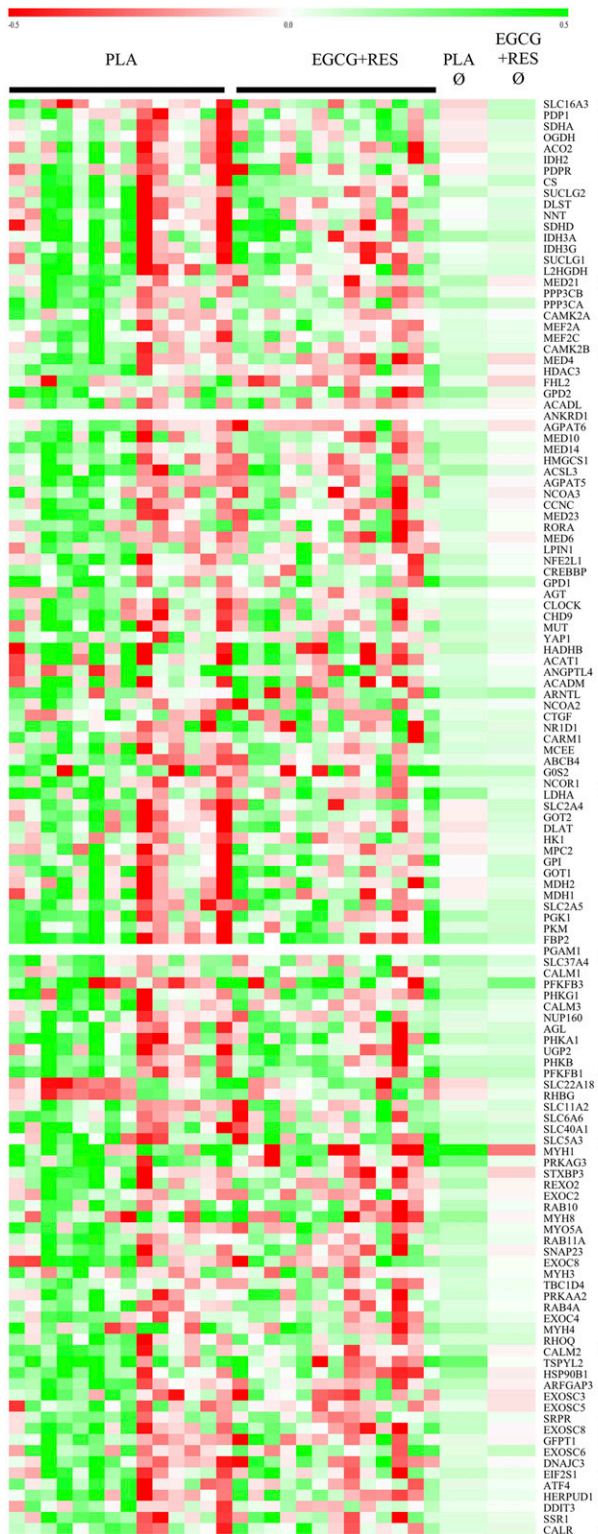


FIGURE 3 Skeletal muscle gene set enrichment analyses before and after the intervention. Heat maps of pathways related to metabolic function from gene set enrichment analyses on skeletal muscle (*Vastus lateralis*) are shown ($n = 27$). Signal log ratios are presented per subject and as means per group. Genes in the heat maps represent significantly contributing genes to the most affected pathways by EGCG+RES or placebo supplementation (false-discovery rate, q -value < 0.2). Colors reflect the signal log ratios per subject and group, with red being downregulated and green being upregulated comparing post- with premeasurement. (A) WP2766: The citric acid TCA cycle and respiratory electron transport. (B) BIOC_PGC1A path-

were not significantly affected by the intervention (**Supplemental Figure 1**). Interstitial glycerol concentrations, which reflect local lipolysis, were also not affected by EGCG+RES (**Supplemental Figure 2**). Furthermore, no effects of EGCG+RES supplementation were observed on interstitial glucose, pyruvate, and lactate concentrations.

EGCG+RES did not affect intramuscular lipid content and composition

Alterations in fat oxidation may contribute to changes in intramyocellular lipid metabolites and, consequently, insulin sensitivity (2). EGCG+RES supplementation for 12 wk did not alter total lipid content (triacylglycerol and diacylglycerol) or saturation of the triacylglycerol and diacylglycerol fractions (**Supplemental Table 2**).

EGCG+RES did not affect tissue-specific insulin sensitivity

Finally, we investigated whether the improved mitochondrial capacity and whole-body fat oxidation resulted in improved insulin sensitivity after EGCG+RES supplementation. We found no changes in peripheral, hepatic, or adipose tissue insulin sensitivity after EGCG+RES supplementation compared with placebo supplementation (**Table 4**), which is in agreement with unchanged fasting and postprandial plasma glucose and insulin concentrations. More specifically, the R_d and EGP under fasting and insulin-stimulated conditions were not significantly affected after 12 wk of EGCG+RES supplementation (**Table 4**). Similar results were found when values were expressed as changes relative to fasting conditions (**Table 4**). Interestingly, EGCG+RES increased fasting NOGD (P -time \times treatment = 0.10, P -EGCG+RES = 0.01, P -placebo = 0.91). Furthermore, EGCG+RES did not improve insulin-mediated suppression of plasma FFAs, indicating that adipose tissue insulin sensitivity was also not affected by EGCG+RES supplementation (P -time \times treatment = 0.63; **Table 4**).

DISCUSSION

Combining polyphenols with distinct mechanisms of action may lead to additional or synergistic beneficial metabolic effects compared with single-polyphenol supplementation (20, 21). Here, we showed that after 12 wk, combined EGCG+RES supplementation preserved fasting and postprandial fat oxidation compared with placebo. This coincided with increased skeletal muscle oxidative capacity in the EGCG+RES group. In addition, EGCG+RES supplementation attenuated the increase in triacylglycerol concentrations compared with placebo, and the reduction in visceral adipose tissue mass tended to be higher with EGCG+RES than with placebo. These beneficial metabolic effects, however, did not translate into improved peripheral, hepatic, and adipose tissue

way. (C) Fatty acid triacylglycerol and ketone body metabolism. (D) WP534: Glycolysis and gluconeogenesis. (E) WP1848: Metabolism of carbohydrates. (F) WP1935: Transport of glucose and other sugars, bile, salts and organic acids, metal, ions, and amine compounds. (G) Translocation of GLUT4 to the plasma membrane. (H) Unfolded protein response. A list of definition of gene and protein names used in this figure is included in the **Supplemental Material**. EGCG+RES, epigallocatechin-3-gallate plus resveratrol; PLA, placebo.

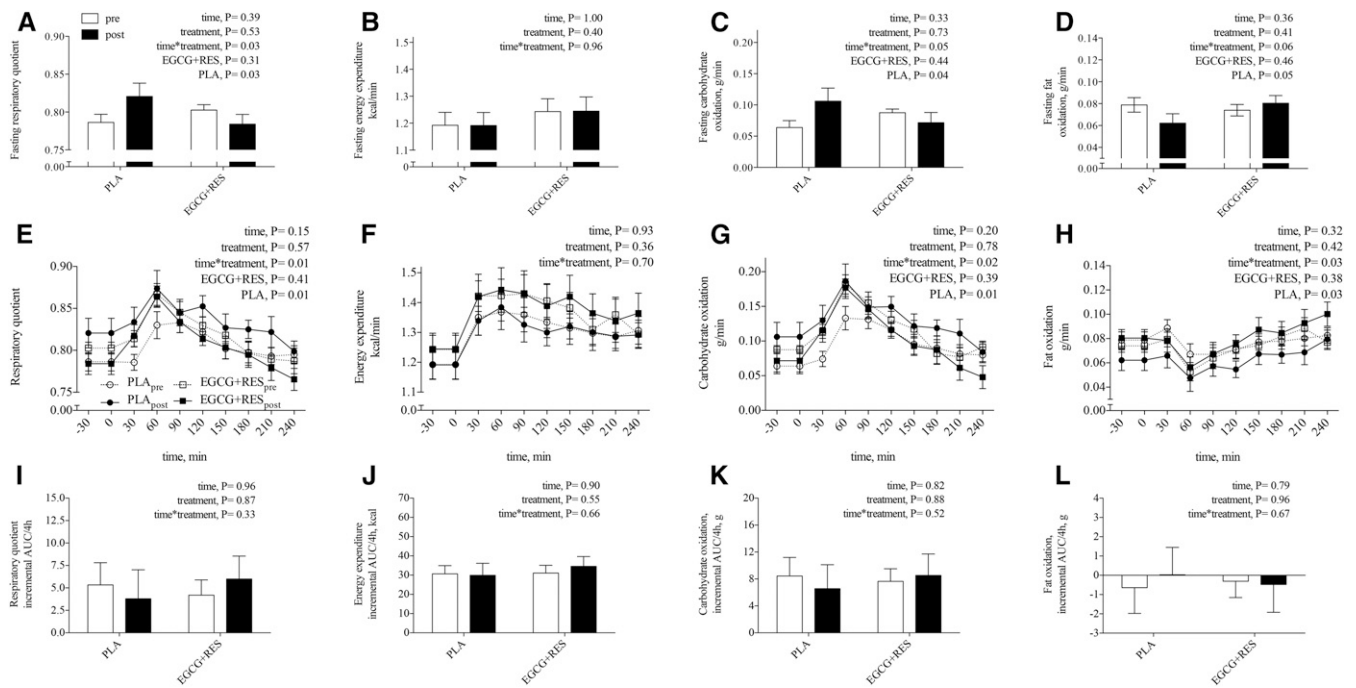


FIGURE 4 Fasting and postprandial whole-body substrate oxidation and energy expenditure before and after the intervention. Values are means \pm SEMs; $n = 31$. Indirect calorimetry was performed during fasting conditions and for 4 h after intake of a liquid HFMM. Mean oxygen consumption and carbon dioxide production over 20 min were used for calculations. Substrate oxidation and energy expenditure were measured after an overnight fast (from -30 to 0 min) and after an HFMM (from 0 to 240 min). Fasting values (A–D), AUCs during the HFMM test (calculated from $t = 0$ to 240 min; E–H), and incremental AUCs (I–L) were analyzed by using 2-factor repeated-measures ANOVA, with time [P -time (pre, post)] and treatment [P -treatment (placebo, EGCG+RES)] as factors. $P < 0.05$ was considered significant. In case of a significant time \times treatment interaction (P -time \times treatment), post hoc analyses with Bonferroni correction were applied to identify significant within-group effects (EGCG+RES, P ; placebo, P). EGCG+RES, epigallocatechin-3-gallate plus resveratrol; HFMM, high-fat mixed meal; PLA, placebo; post, after intervention; pre, before intervention.

insulin sensitivity in overweight men and women. Because the resveratrol supplement is composed of *P. cuspidatum* extract, we cannot exclude metabolic effects of other components [piceid (= resveratrol-glucoside) and emodin (28)].

We previously showed that 3 d of EGCG+RES supplementation increased EE (20). The present study showed that this increase is not maintained after 12 wk of supplementation. Furthermore, consistent with unchanged food intake, we found that body weight and body composition were not affected by longer-term EGCG+RES supplementation. These findings are in line with most (13, 14, 17, 18, 29, 30) but not all (15) previous studies in humans. Although no significant effects on total fat mass were apparent, we found that EGCG+RES tended to decrease visceral adipose tissue mass by $\sim 11\%$ compared with placebo. Likewise, EGCG and resveratrol reduced visceral adipose tissue in animals (31, 32). Because visceral adipose tissue is known to be detrimental to metabolic health (33), its reduction may be of physiologic importance in the long term.

Interestingly, we showed that combined EGCG+RES supplementation significantly affected fasting substrate oxidation compared with placebo (Figure 4). EGCG+RES supplementation preserved fat oxidation, whereas fat oxidation significantly declined in the placebo group. This effect was maintained during postprandial conditions. In agreement with our findings, previous studies in rodents showed that polyphenols may stimulate fat oxidation under fasting conditions (9, 31, 34). The compensatory increase in carbohydrate oxidation that we found in the placebo

group was not accompanied by alterations in whole-body glucose disposal. Rather, we found that EGCG+RES may diminish glycolysis and increase glycogen storage (35), as indicated by the increased fasting NOGD ($\sim 40\%$; P -EGCG+RES = 0.01). Importantly, the differentially affected fat oxidation in the present study is neither driven by an increased lipid supply toward skeletal muscle nor by a significant reduction in muscle lipid stores (triacylglycerol and diacylglycerol content). Indeed, it was previously shown that a shift in fasting fat oxidation can occur independently of changes in intramuscular lipid content, EE, adiposity, or insulin sensitivity (36, 37). Alternatively, a higher oxidative capacity of skeletal muscle mitochondria was found to be predictive of a lower fasting respiratory exchange ratio or, in other words, a greater relative reliance on lipids instead of carbohydrate as a fuel source during fasting conditions (37–39). Interestingly, we found that 12 wk of EGCG+RES supplementation increased skeletal muscle oxidative capacity in permeabilized muscle fibers. This was accompanied by an increased muscle protein content of OxPhos complexes and an upregulation of mitochondrial pathways (citric acid cycle and respiratory electron transport chain) (Figures 2 and 3). In line with these findings, we and others previously showed that resveratrol increased mitochondrial capacity by activating the AMPK-SIRT-PGC1 α pathway (7, 8, 13). It has been suggested that EGCG increases β -adrenergic stimulation by norepinephrine due to catechol-*O*-methyltransferase inhibition (10, 40). However, in our previous study we did not observe altered norepinephrine concentrations after EGCG+RES supplementation (20). Finally,

TABLE 4Whole-body substrate kinetics before and after 12 wk of supplementation with EGCG+RES or placebo¹

	Placebo (<i>n</i> = 19)		EGCG+RES (<i>n</i> = 17)		<i>P</i>		
	Week 0	Week 12	Week 0	Week 12	Time	Treatment	Time × treatment
R_d, μmol/(kg × min)							
Fasting	10.6 ± 0.3	10.7 ± 0.3	10.4 ± 0.2	10.9 ± 0.3	0.25	0.91	0.19
Clamp	31.2 ± 2.3	32.5 ± 2.3	34.2 ± 2.8	35.8 ± 3.3	0.25	0.38	0.89
Stimulation (Δ)	20.6 ± 2.2	21.5 ± 2.3	24.3 ± 3.0	25.0 ± 3.2	0.35	0.27	0.84
NOGD, μmol/(kg × min)							
Fasting	6.1 ± 0.7	6.2 ± 0.6	5.7 ± 0.6	7.9 ± 0.7*	0.05	0.41	0.10
Clamp	20.5 ± 2.4	23.4 ± 1.9	23.0 ± 2.5	25.3 ± 3.1	0.16	0.73	0.95
Stimulation (Δ)	14.4 ± 2.1	17.2 ± 2.1	17.5 ± 2.7	17.4 ± 2.6	0.54	0.76	0.44
EGP							
Fasting, μmol/(kg × min)	10.4 ± 0.3	10.4 ± 0.3	10.1 ± 0.2	10.7 ± 0.3	0.25	0.91	0.19
Clamp, μmol/(kg × min)	4.0 ± 0.6	4.2 ± 0.6	4.6 ± 0.5	4.6 ± 0.5	0.78	0.57	0.98
Suppression, %	60.9 ± 4.9	58.6 ± 4.9	54.1 ± 5.1	56.3 ± 4.7	0.98	0.57	0.74
Plasma FFAs							
Fasting, μmol/L	545 ± 40	547 ± 44	537 ± 42	543 ± 47	0.88	0.91	0.94
Clamp, μmol/L	196 ± 25	210 ± 22	189 ± 26	203 ± 23	0.17	0.83	0.94
Suppression, %	57.7 ± 6.6	57.1 ± 5.2	63.4 ± 7.0	59.0 ± 5.5	0.52	0.62	0.63
Substrate oxidation							
RQ, Δ	0.09 ± 0.01	0.08 ± 0.02	0.10 ± 0.01	0.11 ± 0.01	0.96	0.30	0.31
Carbohydrate, Δ g/min	0.10 ± 0.01	0.09 ± 0.02	0.11 ± 0.01	0.12 ± 0.02	0.93	0.27	0.54
Fat, %	55.5 ± 7.6	54.3 ± 9.1	46.7 ± 6.9	48.4 ± 8.3	0.95	0.47	0.81

¹Values are means ± SEMs. Data were analyzed by using 2-factor repeated-measures ANOVA, with time [*P*-time (pre, post)] and treatment [*P*-treatment (placebo, EGCG+RES)] as factors. *P* < 0.05 was considered significant. No baseline differences were observed (Student's *t* test). *, In case of a significant time × treatment interaction (*P*-time × treatment), post hoc analyses with Bonferroni correction were applied to identify significant within-group effects. EGCG+RES, epigallocatechin-3-gallate plus resveratrol; EGP, endogenous glucose production; FFA, free fatty acid; NOGD, nonoxidative glucose disposal; post, after intervention; pre, before intervention; R_d, rate of disappearance; RQ, respiratory quotient; Δ, absolute difference between fasting and insulin-stimulated conditions.

reduced oxidative stress, indicated by a reduced unfolded protein response pathway (41), might have contributed to the observed improvement in skeletal muscle oxidative capacity (42–44) or vice versa (45).

An increased mitochondrial capacity might reflect an improved metabolic risk profile, because increased mitochondrial capacity has been associated with higher insulin sensitivity in several cross-sectional studies in men (37–39, 46). Therefore, we hypothesized that the improvements in lipid metabolism may lead to increased insulin sensitivity after 12 wk of EGCG+RES supplementation. However, we did not find a significant change in peripheral, hepatic, or adipose tissue insulin sensitivity. To date, only 3 human clinical trials with resveratrol, but not a single study with EGCG, examined the effects of polyphenol supplementation on peripheral and hepatic insulin sensitivity by using the gold-standard hyperinsulinemic-euglycemic clamp. In accordance with our findings, these studies did not show significant changes in peripheral (17, 18, 29) and hepatic (18) insulin sensitivity in lean (18) and obese (17) patients or in patients with nonalcoholic fatty liver disease (29) after supplementation with resveratrol for 4, 8, or 12 wk, respectively. Importantly, in these studies mitochondrial capacity was not assessed (29) or did not improve (17, 18) on the basis of gene expression and protein abundance of related enzymes. Therefore, the present study is the first, to our knowledge, to show that combined EGCG+RES supplementation has beneficial effects on skeletal muscle oxidative capacity, although this was not accompanied by increased peripheral, hepatic, or adipose tissue insulin sensitivity in overweight and obese men and women.

In line with these findings, the insulin-mediated stimulation of carbohydrate oxidation and suppression of lipid oxidation were not affected by EGCG+RES supplementation after meal ingestion or during the hyperinsulinemic-euglycemic clamp. Previously, we showed that single supplementation of resveratrol for 4 wk and combined EGCG+RES supplementation for 3 d improved metabolic flexibility in humans (13, 20), as shown by a more pronounced suppression of postprandial fat oxidation. The apparent discrepancy between the present and earlier studies may be explained by the duration of supplementation. EGCG+RES supplementation had no effect on postprandial glucose, insulin, and FFA concentrations or local interstitial glucose and glycerol concentrations. This is in agreement with other placebo-controlled trials in whites (13, 19), whereas sex-specific analyses may show metabolic effects (14).

Strikingly, we found that EGCG+RES supplementation for 12 wk attenuated the increase in plasma triacylglycerol during the HFMM test that was seen in the placebo group. Previous studies in humans (13, 14) and rodents (9, 31, 47) also showed reduced plasma triacylglycerol after EGCG or resveratrol supplementation. This might, at least in part, be explained by a reduced intestinal lipid uptake, an improved buffering capacity of dietary triacylglycerol in adipose tissue, or increased peripheral (e.g., muscle and liver) triacylglycerol extraction. In accordance, a reduced intestinal lipid uptake after EGCG supplementation in rodents has been suggested (9), although this could not be confirmed in humans (30). In addition, EGCG+RES supplementation may have affected hepatic lipid clearance, storage, and oxidation, as indicated by several animal studies (47–49).

In the present study, we analyzed EGCG, resveratrol, and dihydro-resveratrol plasma concentrations before and after intervention. In future studies, a more detailed characterization of polyphenol metabolites might provide additional insight into polyphenol-induced effects on oxidative metabolism, because polyphenolic metabolites may exert distinct effects on peripheral tissues (50, 51). Furthermore, due to the extensive phenotyping in the current controlled trial, multiple statistical comparisons were made, which may increase the likelihood of obtaining false-positive results. Nevertheless, the fact that our major findings on variables of mitochondrial oxidative capacity and proteins in oxidative phosphorylation and fat oxidation all point toward the same direction of an improvement with EGCG+RES compared with placebo makes it highly unlikely that these are chance findings. Third, in this randomized trial, the statistical plan included a completers analysis taking a drop-out rate of 20% into account, which was higher than the actual drop-out rate. The drop-out rate was similar in both groups (10%; $n = 2$) and was therefore assumed to be randomly distributed. An intention-to-treat analysis would therefore make the reported physiologic effects on oxidative metabolism and fat oxidation only slightly less pronounced.

In conclusion, the present study showed that 12 wk of EGCG+RES supplementation improved skeletal muscle oxidative capacity, preserved fasting and postprandial fat oxidation, and prevented an increase in triacylglycerol concentrations compared with placebo. These putative beneficial metabolic effects did not translate into improved peripheral, hepatic, or adipose tissue insulin sensitivity after 12 wk. Importantly, the improved mitochondrial capacity and fat oxidation may improve physical condition (52) and play a role in the prevention of weight gain (53) and the worsening of insulin resistance (4) in the long term.

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REFERENCES

- Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014;384:766–81.
- Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *N Engl J Med* 2014;371:1131–41.
- Goossens GH. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. *Physiol Behav* 2008;94:206–18.
- Szendroedi J, Phielix E, Roden M. The role of mitochondria in insulin resistance and type 2 diabetes mellitus. *Nat Rev Endocrinol* 2011;8:92–103.
- van Hees AM, Jans A, Hul GB, Roche HM, Saris WH, Blaak EE. Skeletal muscle fatty acid handling in insulin resistant men. *Obesity (Silver Spring)* 2011;19:1350–9.
- Roumen C, Corpeleijn E, Feskens EJ, Mensink M, Saris WH, Blaak EE. Impact of 3-year lifestyle intervention on postprandial glucose metabolism: the SLIM study. *Diabet Med* 2008;25:597–605.
- Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 2006;444:337–42.
- Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 α . *Cell* 2006;127:1109–22.
- Klaus S, Pultz S, Thone-Reineke C, Wolfram S. Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. *Int J Obes (Lond)* 2005;29:615–23.
- Lu H, Meng X, Yang CS. Enzymology of methylation of tea catechins and inhibition of catechol-O-methyltransferase by (-)-epigallocatechin gallate. *Drug Metab Dispos* 2003;31:572–9.
- Collins QF, Liu HY, Pi J, Liu Z, Quon MJ, Cao W. Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, suppresses hepatic gluconeogenesis through 5'-AMP-activated protein kinase. *J Biol Chem* 2007;282:30143–9.
- Bogdanski P, Suliburska J, Szulinska M, Stepień M, Pupek-Musialik D, Jabłeczka A. Green tea extract reduces blood pressure, inflammatory biomarkers, and oxidative stress and improves parameters associated with insulin resistance in obese, hypertensive patients. *Nutr Res* 2012;32:421–7.
- Timmers S, Konings E, Bilet L, Houtkooper RH, van de Weijer T, Goossens GH, Hoeks J, van der Krieken S, Ryu D, Kersten S, et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab* 2011;14:612–22.
- Mähler A, Steiner J, Bock M, Klug L, Parreidt N, Lorenz M, Zimmermann BF, Krannich A, Paul F, Boschmann M. Metabolic response to epigallocatechin-3-gallate in relapsing-remitting multiple sclerosis: a randomized clinical trial. *Am J Clin Nutr* 2015;101:487–95.
- Brown AL, Lane J, Holyoak C, Nicol B, Mayes AE, Dadd T. Health effects of green tea catechins in overweight and obese men: a randomised controlled cross-over trial. *Br J Nutr* 2011;106:1880–9.
- Mendez-Del Villar M, Gonzalez-Ortiz M, Martinez-Abundis E, Perez-Rubio KG, Lizarraga-Valdez R. Effect of resveratrol administration on metabolic syndrome, insulin sensitivity, and insulin secretion. *Metab Syndr Relat Disord* 2014;12:497–501.
- Poulsen MM, Vestergaard PF, Clasen BF, Radko Y, Christensen LP, Stodkilde-Jorgensen H, Møller N, Jessen N, Pedersen SB, Jorgensen JO. High-dose resveratrol supplementation in obese men: an investigator-initiated, randomized, placebo-controlled clinical trial of substrate metabolism, insulin sensitivity, and body composition. *Diabetes* 2013;62:1186–95.
- Yoshino J, Conte C, Fontana L, Mittendorfer B, Imai S, Schechtman KB, Gu C, Kunz I, Rossi Fanelli F, Patterson BW, et al. Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. *Cell Metab* 2012;16:658–64.
- Brown AL, Lane J, Coverly J, Stocks J, Jackson S, Stephen A, Bluck L, Coward A, Hendrickx H. Effects of dietary supplementation with the green tea polyphenol epigallocatechin-3-gallate on insulin resistance and associated metabolic risk factors: randomized controlled trial. *Br J Nutr* 2009;101:886–94.
- Most J, Goossens GH, Jocken JW, Blaak EE. Short-term supplementation with a specific combination of dietary polyphenols increases energy expenditure and alters substrate metabolism in overweight subjects. *Int J Obes (Lond)* 2014;38:698–706.
- Van Can J, Van Dijk J-W, Goossens G, Jocken J, Hospers J, Blaak EA. 3 day EGCG supplementation reduced lactate in skeletal muscle in overweight subjects. *Obes Rev* 2010;11:206.
- Phielix E, Schrauwen-Hinderling VB, Mensink M, Lenaers E, Meex R, Hoeks J, Kooi ME, Moonen-Kornips E, Sels JP, Hesselink MK, et al. Lower intrinsic ADP-stimulated mitochondrial respiration underlies in vivo mitochondrial dysfunction in muscle of male type 2 diabetic patients. *Diabetes* 2008;57:2943–9.
- Goossens GH, Blaak EE, Saris WH, van Baak MA. Angiotensin II-induced effects on adipose and skeletal muscle tissue blood flow and lipolysis in normal-weight and obese subjects. *J Clin Endocrinol Metab* 2004;89:2690–6.
- Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 1949;109:1–9.
- Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* 1983;55:628–34.

26. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
27. Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N Engl J Med* 1991;325:373–81.
28. Kuo CH, Chen BY, Liu YC, Chang CMJ, Deng TS, Chen JH, Shieh CJ. Optimized ultrasound-assisted extraction of phenolic compounds from *Polygonum cuspidatum*. *Molecules* 2013;19:67–77.
29. Chachay VS, Macdonald GA, Martin JH, Whitehead JP, O'Moore-Sullivan TM, Lee P, Franklin M, Klein K, Taylor PJ, Ferguson M, et al. Resveratrol does not benefit patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2014.
30. Janssens PL, Hursel R, Westerterp-Plantenga MS. Long-term green tea extract supplementation does not affect fat absorption, resting energy expenditure, and body composition in adults. *J Nutr* 2015;145:864–70.
31. Friedrich M, Petzke KJ, Raederstorff D, Wolfram S, Klaus S. Acute effects of epigallocatechin gallate from green tea on oxidation and tissue incorporation of dietary lipids in mice fed a high-fat diet. *Int J Obes (Lond)* 2012;36:735–43.
32. Jimenez-Gomez Y, Mattison JA, Pearson KJ, Martin-Montalvo A, Palacios HH, Sossong AM, Ward TM, Younts CM, Lewis K, Allard JS, et al. Resveratrol improves adipose insulin signaling and reduces the inflammatory response in adipose tissue of rhesus monkeys on high-fat, high-sugar diet. *Cell Metab* 2013;18:533–45.
33. Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. *Physiol Rev* 2013;93:359–404.
34. Park SJ, Ahmad F, Philp A, Baar K, Williams T, Luo H, Ke H, Rehmann H, Taussig R, Brown AL, et al. Resveratrol ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases. *Cell* 2012;148:421–33.
35. Bouskila M, Hunter RW, Ibrahim AF, Delattre L, Pegg M, van Diepen JA, Voshol PJ, Jensen J, Sakamoto K. Allosteric regulation of glycogen synthase controls glycogen synthesis in muscle. *Cell Metab* 2010;12:456–66.
36. Hoehn KL, Turner N, Swarbrick MM, Wilks D, Preston E, Phua Y, Joshi H, Furler SM, Larance M, Hegarty BD, et al. Acute or chronic upregulation of mitochondrial fatty acid oxidation has no net effect on whole-body energy expenditure or adiposity. *Cell Metab* 2010;11:70–6.
37. van de Weijer T, Sparks LM, Phielix E, Meex RC, van Herpen NA, Hesselink MK, Schrauwen P, Schrauwen-Hinderling VB. Relationships between mitochondrial function and metabolic flexibility in type 2 diabetes mellitus. *PLoS One* 2013;8:e51648.
38. Phielix E, Meex R, Ouwens DM, Sparks L, Hoeks J, Schaart G, Moonen-Kornips E, Hesselink MK, Schrauwen P. High oxidative capacity due to chronic exercise training attenuates lipid-induced insulin resistance. *Diabetes* 2012;61:2472–8.
39. Corpeleijn E, Mensink M, Kooi ME, Roekaerts PM, Saris WH, Blaak EE. Impaired skeletal muscle substrate oxidation in glucose-intolerant men improves after weight loss. *Obesity (Silver Spring)* 2008;16:1025–32.
40. Hursel R, Janssens PL, Bouwman FG, Mariman EC, Westerterp-Plantenga MS. The role of catechol-O-methyl transferase Val(108/158) Met polymorphism (rs4680) in the effect of green tea on resting energy expenditure and fat oxidation: a pilot study. *PLoS One* 2014;9:e106220.
41. Zhang K, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. *Nature* 2008;454:455–62.
42. Jackson JR, Ryan MJ, Alway SE. Long-term supplementation with resveratrol alleviates oxidative stress but does not attenuate sarcopenia in aged mice. *J Gerontol A Biol Sci Med Sci* 2011;66:751–64.
43. Serrano JC, Gonzalo-Benito H, Jove M, Fourcade S, Cassanye A, Boada J, Delgado MA, Espinel AE, Pamplona R, Portero-Otin M. Dietary intake of green tea polyphenols regulates insulin sensitivity with an increase in AMP-activated protein kinase alpha content and changes in mitochondrial respiratory complexes. *Mol Nutr Food Res* 2013;57:459–70.
44. Boudina S, Sena S, Sloan C, Tebbi A, Han YH, O'Neill BT, Cooksey RC, Jones D, Holland WL, McClain DA, et al. Early mitochondrial adaptations in skeletal muscle to diet-induced obesity are strain dependent and determine oxidative stress and energy expenditure but not insulin sensitivity. *Endocrinology* 2012;153:2677–88.
45. Liesa M, Shrihail OS. Mitochondrial dynamics in the regulation of nutrient utilization and energy expenditure. *Cell Metab* 2013;17:491–506.
46. Ukropcova B, McNeil M, Sereda O, de Jonge L, Xie H, Bray GA, Smith SR. Dynamic changes in fat oxidation in human primary myocytes mirror metabolic characteristics of the donor. *J Clin Invest* 2005;115:1934–41.
47. Cho SJ, Jung UJ, Choi MS. Differential effects of low-dose resveratrol on adiposity and hepatic steatosis in diet-induced obese mice. *Br J Nutr* 2012;108:2166–75.
48. Zhou J, Farah BL, Sinha RA, Wu Y, Singh BK, Bay BH, Yang CS, Yen PM. Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, stimulates hepatic autophagy and lipid clearance. *PLoS One* 2014;9:e87161.
49. Murase T, Nagasawa A, Suzuki J, Hase T, Tokimitsu I. Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. *Int J Obes Relat Metab Disord* 2002;26:1459–64.
50. Calamini B, Ratia K, Malkowski MG, Cuendet M, Pezzuto JM, Santarsiero BD, Mesecar AD. Pleiotropic mechanisms facilitated by resveratrol and its metabolites. *Biochem J* 2010;429:273–82.
51. Lambert JD, Sang S, Yang CS. Biotransformation of green tea polyphenols and the biological activities of those metabolites. *Mol Pharm* 2007;4:819–25.
52. Overmyer KA, Evans CR, Qi NR, Minogue CE, Carson JJ, Chermide-Scabbo CJ, Koch LG, Britton SL, Pagliarini DJ, Coon JJ, et al. Maximal oxidative capacity during exercise is associated with skeletal muscle fuel selection and dynamic changes in mitochondrial protein acetylation. *Cell Metab* 2015;21:468–78.
53. Flatt JP, Ravussin E, Acheson KJ, Jequier E. Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. *J Clin Invest* 1985;76:1019–24.